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Crystallization and preliminary X-ray analysis of Rv1674c from *Mycobacterium tuberculosis*

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Adaptations to hypoxia play an important role in *Mycobacterium tuberculosis* pathogenesis. Rv0324, which contains an HTH DNA-binding domain and a rhodanese domain, is one of the key transcription regulators in response to hypoxia. *M. tuberculosis* Rv1674c is a homologue of Rv0324. To understand the interdomain interaction and regulation of the HTH domain and the rhodanese domain, recombinant Rv1674c protein was purified and crystallized by the vapour-diffusion method. The crystals diffracted to 2.25 Å resolution. Preliminary diffraction analysis suggests that the crystals belonged to space group $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 67.8$, $c = 174.5$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. The Matthews coefficient was calculated to be 2.44 Å^3 Da^{-1}, assuming that the crystallographic asymmetric unit contains two protein molecules.

1. Introduction

Tuberculosis (TB) is a challenging problem that threatens the health of much of the world's population. The detection and the treatment of TB is complicated because *Mycobacterium tuberculosis* (MTB) can survive within the host for months to decades in an asymptomatic state (Manabe & Bishai, 2000; Flynn & Chan, 2001). The mechanism of persistence of MTB is far from clearly understood.

Recently, the MTB regulatory network based on 50 transcription factors has been described and has provided new insight into the physiological consequences of the regulatory programs induced by changes in oxygen availability (Galagan *et al.*, 2013). Adaptations to hypoxia are thought to play a prominent role in the persistence of MTB. In this network, Rv0324 is an important knot. It is predicted to activate another knot, KstR, which is a cholesterol degradation repressor. Rv0324 and KstR are both associated with the enduring hypoxic response (EHR), which is induced later but not in the initial response to hypoxia (Rustad *et al.*, 2008). Rv0324 contains two domains: an N-terminal HTH ArsR-type DNA-binding domain and a C-terminal rhodanese domain. Sequence analysis shows that Rv0324 only shares sequence homology with Rv1674c in MTB. Rv0324 and Rv1674c share 45% sequence identity and thus are deduced to have similar biochemical functions.

The SmtB/ArsR family proteins are transcriptional repressors that sense distinct metal ions and are involved in the regulation of metal detoxification (Busenlehner *et al.*, 2003). Metal binding by these proteins directly negatively regulates binding of the operator/promoter (O/P) region (Wu & Rosen, 1993). The structures of members of this family reveal that the

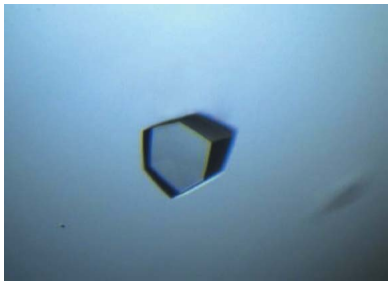

Table 1
Macromolecule-production information.

Source organism	*M. tuberculosis*
DNA source	Synthesized
Expression vector	pET-28a-SUMO
Expression host	*E. coli* strain BL21 (DE3)
Complete amino-acid sequence of the construct produced	MGSSHHHHHHSSGLVPRGSHMASMSDSEVNQEAK-PEVKPEVKPETHINLKVSDGSSEIFFKIKKTT-PLRRLMEAFAKRQGKEMDSLRFLYDGIRIQAD-QTPEDLDMEDNDIIEAHREQIGGSMSGAKKLI-FEQFALVGQALSSGHRLELLDLLVQGERSVDA-LARASGLTFANASQHLLQLRRAGLVTSRRDGK-RVIYALSDPQVWDVVRAVRAVAERNLASVGSL-VRQYYTDRDSLEPISRDELQARVAAGSVLVLD-VRPAMEYAAGHLPGAVSIPLDELAERLDELPS-GIDIVACCRGPYCVYAYDALELLRPNGFSARR-LDGGFSEWLAADLPVVRT

ArsR type of HTH domain often forms a homodimer to bind double-stranded DNA (Lee *et al.*, 2012). On the other hand, rhodaneses are widely distributed enzymes that catalyse the transfer of a sulfane from a sulfur donor such as thiosulfate to a sulfur acceptor such as cyanide (Nandi & Westley, 1998). Rhodanese-like proteins alone or in combination with other proteins perform roles in cyanide detoxification, the restoration of iron–sulfur centres in Fe–S proteins, the management of stress tolerance and the maintainance of redox homeostasis (Ogasawara *et al.*, 2001; Sabelli *et al.*, 2008).

Although the three-dimensioned structures of the HTH domain and the rhodanese domain have been intensively studied, no structure of a combination of these two domains within one protein such as in Rv0324 and Rv1674c has been reported. The functional mechanism of Rv0324/Rv1674c should be related to the interdomain regulation of the HTH and rhodanese domains. In addition, the highest sequence identity of a structurally determined rhodanese to the Rv0324 rhodanese domain is only 35%. More importantly, the sequences of the predicted active regions of Rv0324 and Rv1674c, with two or three cysteine residues, are not comparable to those of the structurally determined rhodanese, which contains only one catalytic residue. To investigate the structure–function relationship of Rv0324/Rv1674c, as well as the interaction or regulation between the HTH domain and rhodanese domain within one protein molecule, we tried to study the crystal structures of Rv0324 and Rv1674c. Unfortunately, attempts to crystallize Rv0324 in our laboratory failed. Here, we report the expression, purification and preliminary crystallographic analysis of Rv1674c. The structure of Rv1674c should provide useful information to understand the regulatory mechanism of Rv0324.

2. Materials and methods

2.1. Macromolecule production

The gene for Rv1674c (UniProt accession No. O53921) was optimized for expression in *Escherichia coli* and synthesized by GenScript (Nanjing, People's Republic of China). It was then subcloned into the pET-28a-SUMO (Novagen) expression vector with XhoI and BamHI restriction sites. An N-terminal His_6 tag and a SUMO tag from the vector were thus fused to Rv1674c (Table 1).

E. coli strain BL21 (DE3) cells were transformed with the recombined plasmid containing Rv1674c. The transformants were cultured in LB medium contaning 25 µg ml^{-1} kanamycin at 310 K until the OD_{600} reached 0.6–0.8. IPTG was then added to the culture medium to a final concentration of 0.2 m*M* for the production of protein and the cells were cultured for a further 4 h at 310 K. The cells were harvested by centrifugation at 4000*g* for 30 min at 277 K.

For the purification of Rv1674c, the cells were resuspended in lysis buffer (50 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl, 1 m*M* PMSF) and lysed by sonication. The lysate was centrifuged at 38 900*g* for 30 min at 277 K. The supernatant was loaded onto 5 ml Ni–NTA matrix (GE Healthcare). The target protein was eluted with lysis buffer containing 200 m*M* imidazole. ULP1 protease was then added to the protein at a mass ratio of 1:20 to remove the SUMO tag. After digestion, the mixture was applied onto Ni–NTA column beads again so that the N-terminal His-SUMO tag remained bound to the beads. The flowthrough containing Rv1674c with no tag was collected and was concentrated by ultrafiltration for gel-filtration chromatography. The protein sample was loaded onto a Superdex 75 column (GE Healthcare) equilibrated with buffer consisting of 50 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl at 277 K. Fractions containing Rv1674c as identified by SDS–PAGE were collected and concentrated for crystallization. The protein concentration was determined by the Bradford method using a Bio-Rad Protein Assay Kit.

2.2. Crystallization

For initial crystallization, the concentration of Rv1674c was adjusted to 7.5 mg ml^{-1}. Crystallization-condition screening was carried out using the sitting-drop vapour-diffusion method at 289 K with the commercial screening kits Crystal Screen, Crystal Screen 2, Index and PEG/Ion from Hampton Research and The Nucleix Suite and The Cryos Suite from Qiagen. 300 nl protein solution was mixed with 300 nl reservoir solution and equilibrated against 35 µl reservoir solution in 96-well plates using a Mosquito robot. Crystals were found in several conditions after a week. To improve the crystal quality, optimization of the crystallization condition was performed using sitting-drop vapour diffusion in 48-well plates and hanging-drop vapour diffusion in 24-well plates with 1 µl protein solution and 1 µl reservoir solution. Different precipitant concentrations, salt concentrations and pH values were used in the reservoir solutions. Different protein concentrations (3.5 and 5 mg ml^{-1}) were also used for further optimization.

2.3. Data collection and processing

Diffraction data were collected on beamline BL17U at Shanghai Synchrotron Radiation Facility (SSRF) using an ADSC Q315 detector at 100 K. Crystals were loop-mounted and flash-cooled in liquid nitrogen. A total of 180 diffraction images were collected with an oscillation of 1° and an exposure time of 0.5 s per image. The diffraction data were

Table 2
Crystallization conditions for the crystal that was used for diffraction data collection.

Method	Hanging-drop vapour diffusion
Plate type	24-well plates
Temperature (K)	289
Protein concentration (mg ml^{-1})	3.5
Buffer composition of protein solution	50 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl
Composition of reservoir solution	0.05 *M* sodium cacodylate pH 6.5, 0.2 *M* KCl, 2.5%(*w*/*v*) hexanediol
Volume and ratio of drop	2 µl drop: 1 µl protein solution and 1 µl reservoir solution
Volume of reservoir (µl)	200

indexed, integrated and scaled using the *HKL*-2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

Recombinant Rv1674c protein from *M. tuberculosis* with an N-terminal His-SUMO tag was successfully produced in *E. coli* cells. Soluble protein was obtained after sonication and was eluted from an Ni–NTA affinity column. The N-terminal His-SUMO tag was cleaved using ULP1 protease and was removed completely after a second Ni-affinity column and gel-filtration purification. A single protein band of approximately 26 kDa was observed by SDS–PAGE and the molecular mass is consistent with the calculated mass of full-length Rv1674c (Fig. 1). The purity of the protein was estimated to be at least 95%. According to the elution volumn of Rv1674c and that of the low-molecular-weight protein marker (GE Healthcare) during gel-filtration chromatography, Rv1674c forms a dimer in solution, which is in agreement with other proteins containing an HTH DNA-binding domain.

The crystals found in the initial screening conditions were small and were not well shaped. Reduction of the protein concentration reduced the number of crystal nuclei and

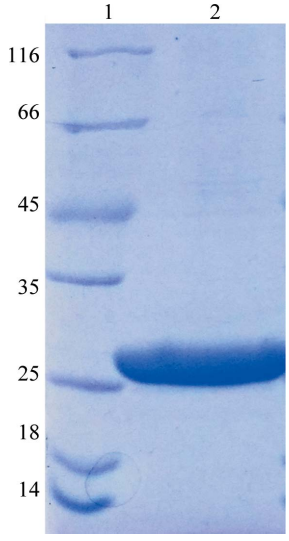

Figure 1
SDS–PAGE of Rv1674c. Lane 1, molecular-weight marker (labelled on the left in kDa); lane 2, purified Rv1674c after gel filtration.

Table 3
Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	SSRF
Wavelength (Å)	0.9789
Temperature (K)	100
Detector	ADSC Q315
Crystal-to-detector distance (mm)	300
Rotation range per image (°)	1
Total rotation range (°)	180
Exposure time per image (s)	0.5
Space group	$P3_121$ or $P3_221$
a, *b*, *c* (Å)	67.8, 67.8, 174.5
α, β, γ (°)	90, 90, 120
Mosaicity (°)	0.4
Resolution range (Å)	50.00–2.25 (2.33–2.25)
Total No. of reflections	218734 (21893)
No. of unique reflections	22847 (2234)
Completeness (%)	100.0 (100.0)
Multiplicity	9.6 (9.8)
$\langle I/\sigma(I)\rangle$	22.1 (5.8)
$R_{\rm merge}$† (%)	9.0 (43.3)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an individual measurement of a reflection and $\langle I(hkl)\rangle$ is the mean value for all equivalent measurements of this reflection.

enlarged the crystals. The crystal used for diffraction data collection appeared in an optimized condition consisting of 0.05 *M* sodium cacodylate pH 6.5, 0.2 *M* KCl, 2.5%(*w*/*v*) hexanediol using the hanging-drop vapour-diffusion method with 3.5 mg ml^{-1} protein at 289 K after a week (Table 2). The crystal shape was hexagonal and the dimensions of the crystal were about 110 × 110 × 50 µm (Fig. 2). The Rv1674c crystal diffracted to 2.25 Å resolution at SSRF. Processing and analysis of the diffraction data indicated that the unit-cell parameters were $a = b = 67.8$, $c = 174.5$ Å, $\alpha = \beta = 90$, $\gamma = 120°$ and that the crystals belonged to space group $P3_121$ or $P3_221$, which share the same diffraction absence pattern and are not distinguishable at the current stage. The data-processing statistics are listed in Table 3. Calculation of the Matthews coefficient suggested that there would be two Rv1674c molecules in the asymmetric unit, with a Matthews coefficient of 2.44 Å^3 Da^{-1} and a solvent content of 50% (Matthews, 1968). Similar structures have been reported for both the HTH DNA-binding domain and the rhodanese domain of Rv1674c,

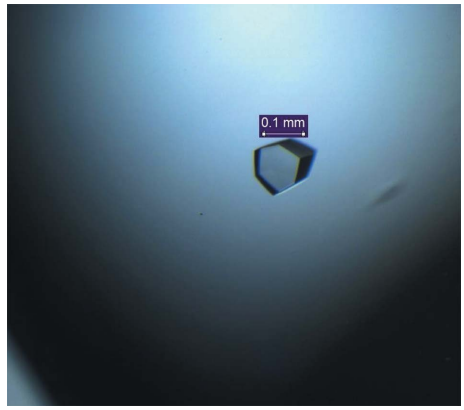

Figure 2
Crystal of Rv1674c.

and it is expected that the structure of Rv1674c will be determined by molecular replacement using these similar structures as search models.

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